```
=> d his

     (FILE 'HOME' ENTERED AT 13:32:15 ON 15 OCT 2005)
     FILE 'CA' ENTERED AT 13:32:23 ON 15 OCT 2005
        E DEXTER D/AU
L1  163 S E3,E5,E9-12,E15-16
L2    4 S L1 AND 1953/PY
L3    1 S L2 AND LUMINE?
        E TYAGI S/AU
L4   12 S E3,E20 AND 1996/PY
L5    1 S L4 AND BIOTECHNOLOGY/SO
L6  908 S (FLUORESCEN? OR FLUORESING)AND((STATIC OR GROUND STATE)(4A)QUENCH?
        OR NONFRET OR NON FRET OR(NONFLUORESCEN? OR NON FLUORESCEN?)(3A)
        COMPLEX)
L7  214 S L6 AND(PROTEIN OR PEPTIDE OR DNA OR RNA OR TRNA OR MRNA OR
        OLIGONUCLEOTIDE OR POLYNUCLEOTIDE OR POLYNUCLEIC OR OLIGOSACCHARIDE
        OR BIOLOGICAL(1A)MOLECULE OR BIOMOLECULE)
L8  134 S L7 NOT PY>1999
L9    2 S L7 NOT L8 AND PATENT/DT AND PY<2001
L10 127 S (FLUORESCEN? OR FLUORESING)AND BEACON AND QUENCH? NOT L6
L11 118 S L10 AND(PROTEIN OR PEPTIDE OR DNA OR RNA OR TRNA OR MRNA OR
        OLIGONUCLEOTIDE OR POLYNUCLEOTIDE OR POLYNUCLEIC OR OLIGOSACCHARIDE
        OR BIOLOGICAL(1A)MOLECULE OR BIOMOLECULE)
L12   6 S L11 NOT PY>1999
L13   7 S L11 NOT L12 AND PATENT/DT AND PY<2001
L14 140 S L8,L12
     FILE 'BIOSIS' ENTERED AT 13:53:39 ON 15 OCT 2005
L15  96 S L14
     FILE 'MEDLINE' ENTERED AT 13:54:09 ON 15 OCT 2005
L16 100 S L14
     FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 13:54:57 ON 15 OCT 2005
L17 183 DUP REM L9 L13 L14 L15 L16 (162 DUPLICATES REMOVED)

=> d bib,ab 1-183

L17  ANSWER 5 OF 183  CA  COPYRIGHT 2005 ACS on STN
AN   130:321569  CA
TI   Linear Beacons containing energy-transferring donors and acceptors and
     their use in nucleic acid hybridization
IN   Gildea, Brian D.; Coull, James M.; Hyldig-Nielsen, Jens J.; Fiandaca,
     Mark J.
PA   Boston Probes, Inc., USA
SO   PCT Int. Appl., 78 pp.
PI   WO 9921881          A1    19990506    WO 1998-US22630        19981027
     <--
     US 6485901          B1    20021126    US 1998-179162         19981026
PRAI US 1997-63283P      P     19971027
AB   This invention is directed to methods, kits and compns. pertaining to
     Linear Beacons.  Linear Beacons are polymers contg. donor and acceptor
     moieties sepd. by a nucleobase sequence.  The polymers do not
     necessarily form a stem-loop hairpin.  The efficiency of energy transfer
     between the donor and acceptor moieties is substantially independent of
     at least two of the following variables: sequence length, spectral
     overlap of donor and acceptor, presence or absence of Mg, and ionic
```

strength of the soln. Preferred linear beacons are fluorophore-contg. **peptide** nucleic acids (PNAs). In the absence of a target sequence, Linear Beacons facilitate efficient energy transfer between the donor and acceptor moieties linked to opposite ends of the probe. Upon hybridization of the probe to a target sequence, there is a measurable change in at least one property of at least one donor or acceptor moiety of the probe which can be used to detect, identify or quantitate the target sequence in a sample. Expts. demonstrating the **non-FRET** behavior of the PNA Linear Beacons and their use in detection of Pseudomonas aeruginosa and Bacillus subtilis as well as detection of PCR-amplified K-ras gene were demonstrated.

L17 ANSWER 8 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 131:54725 CA
TI Homogeneous detection of a target through nucleic acid ligand-ligand **beacon** interaction
IN Jayasena, Sumedha; Gold, Larry
PA Nexstar Pharmaceuticals, Inc., USA
SO PCT Int. Appl., 76 pp.
PI WO 9931276 A1 19990624 WO 1998-US26599 19981215
<--
US 5989823 A 19991123 US 1998-157206 19980918
<--
PRAI US 1997-68135P P 19971215
AB A homogeneous assay that utilizes mol. **beacons** as the reporter and nucleic acid ligands as the sensor is described. This assay, called the ligand **beacon** assay, is for the detection of target mols. in a test mixt. The concept of the ligand **beacon** assay was tested using several **proteins** to which high affinity and specific nucleic acid ligands are available. The assay specifically detects the mol. target that binds the nucleic acid ligand with high affinity and specificity. The range of the assay is dictated by the concn. of the nucleic acid ligand/ligand **beacon** pair used in the assay. Target **proteins** were detected in buffer as well as in plasma, expanding its applicability to clin. use. This is a simple to use and fast assay format with the potential for automation for high throughput screening applications.

L17 ANSWER 13 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 132:10457 CA
TI New Donor-Acceptor Pair for **Fluorescent** Immunoassays by Energy Transfer
AU Schobel, Uwe; Egelhaaf, Hans-Joachim; Brecht, Andreas; Oelkrug, Dieter; Gauglitz, Guenter
CS Institute of Physical and Theoretical Chemistry, Tuebingen, D-72076, Germany
SO Bioconjugate Chemistry (1999), 10(6), 1107-1114
AB A novel Forster donor-acceptor dye pair for an immunoassay based on resonance energy transfer (RET) is characterized with respect to its photophys. properties. As donor and acceptor, we chose the long-wavelength excitable cyanine dyes Cy5 and Cy5.5, resp. Due to the perfect spectral overlap, an exceptionally high R0 value of 68.7 Å is obtained in soln. For biochem. applications, antibodies (IgG) are labeled with Cy5, while a tracer for competitive binding is synthesized by labeling bovine serum albumin (BSA) with an analyte deriv. and Cy5.5.

Binding the dyes to **proteins** at a low dye/**protein** ratio increases the **fluorescence** lifetimes and quantum yields, leading to an enhanced R0 value of 85.2 Å. At higher dye/**protein** ratios, the formation of nonfluorescent dimeric species causes a decrease in the **fluorescence** lifetime and quantum yield due to RET from monomeric dyes to dimers within one **protein** mol. The Forster distances could be calcd. using the dimer absorption spectra to 83.9 and 83.6 Å for Cy5 and Cy5.5, resp. Upon binding of the Cy5-labeled IgG to the tracer, efficient quenching of Cy5 **fluorescence** is obsd. Steady-state and time-resolved measurements reveal that approx. 50% of the quenching results in Forster-type RET, while the residual **quenching** effect is caused by **static quenching** processes. The applicability of this dye pair is demonstrated in a homogeneous competitive immunoassay for the pesticide simazine.

L17 ANSWER 26 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 129:60111 CA
TI The correct use of "average" **fluorescence** parameters
AU Sillen, Alain; Engelborghs, Yves
CS Laboratory of Chemical and Biological Dynamics, Katholieke Universiteit Leuven, Louvain, B-3001, Belg.
SO Photochemistry and Photobiology (1998), 67(5), 475-486
AB When more than one fluorophore is present or when one fluorophore displays a multiple exponential decay av. **fluorescence** parameters are derived, which can be combined with av. lifetimes for further interpretation. However, two kinds of av. lifetimes were used in this context: the intensity and the amplitude av. lifetime. The different av. parameters are carefully analyzed and their best combinations are derived. These av. parameters are analyzed in the context of external and internal dynamic and **static quenching**, Foster energy transfer and the calcn. of the radiative rate const. The use of the amplitude av. lifetime for the anal. of multiple fluorophore-contg. systems and the detection of interactions is discussed.

L17 ANSWER 29 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 129:286496 CA
TI PNA molecular **beacons** for rapid detection of PCR amplicons
AU Ortiz, E.; Estrada, G.; Lizardi, P. M.
CS Department of Molecular Recognition and Structural Biology, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos, 62271, Mex.
SO Molecular and Cellular Probes (1998), 12(4), 219-226
AB The authors have developed a method for rapid detection of polymerase chain reaction (PCR) amplicons based on surface immobilized PNA-**DNA** hybrid probes ('mol. **beacons**') that undergo a **fluorescent**-linked conformational change in the presence of a complementary **DNA** target. Amplicons can be detected by simply adding a PCR reaction to a microtiter-well contg. the previously immobilized probe, and reading the generated **fluorescence**. No further transfers or washing steps are involved. The authors demonstrate the specificity of the method for the detection of ribosomal **DNA** from Entamoeba histolytica.

L17 ANSWER 32 OF 183 CA COPYRIGHT 2005 ACS on STN

AN 128:163303 CA
TI Multicolor molecular **beacons** for allele discrimination
AU Tyagi, Sanjay; Bratu, Diana P.; Kramer, Fred Russell
CS Dep. Molecular Genetics, Public Health Res. Inst., New York, NY, 10016, USA
SO Nature Biotechnology (1998), 16(1), 49-58
AB Mol. **beacons** are hairpin-shaped **oligonucleotide** probes that report the presence of specific nucleic acids in homogeneous solns. When they bind to their targets they undergo a conformational reorganization that restores the **fluorescence** of an internally **quenched** fluorophore. We found that their hairpin conformation enables the use of a wide variety of differently colored fluorophores. Using several mol. **beacons**, each designed to recognize a different target and each labeled with a different fluorophore, we demonstrate that multiple targets can be distinguished in the same soln., even if they differ from one another by as little as a single nucleotide. A comparison of "hairpin probes" with corresponding "linear probes" confirms that the presence of the hairpin stem in mol. **beacons** significantly enhances their specificity.

L17 ANSWER 35 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 128:150731 CA
TI **Fluorescence** properties of a new guanosine analog incorporated into small **oligonucleotides**
AU Driscoll, Sarah L.; Hawkins, Mary E.; Balis, Frank M.; Pfleiderer, Wolfgang; Laws, William R.
CS Department of Biochemistry, Mount Sinal School of Medicine, New York, NY, 10029, USA
SO Biophysical Journal (1997), 73(6), 3277-3286
AB The **fluorescence** properties of 3-methyl-isoxanthopterin (3-MI) incorporated into different **oligonucleotides** have been detd. This highly **fluorescent** guanosine analog has its absorption and **fluorescence** spectra well resolved from those of the normal nucleotides and the arom. amino acids. The small shifts obsd. in absorption and **fluorescence** emission spectra upon incorporation of 3-MI into these **oligonucleotides** are consistent with a general solvent effect and do not suggest any contribution from the position of the probe from the 5' end, the sequence of nucleotides immediately 5' or 3' to the probe, or the single- or double-stranded nature of the oligomer. However, steady-state and time-resolved **fluorescence** studies indicate that the presence of a purine immediately 5' or 3' to the probe results in some dynamic but mostly **static quenching** in the single-stranded oligomer. Furthermore, a 3' purine is more effective than a 5' purine, and an adenine appears to be more effective than a guanine for these **static quenching** interactions. Formation of the double-stranded oligomer leads to an addnl. loss of quantum yield, which can also be ascribed primarily to **static quenching**. These results show that this new class of spectrally enhanced **fluorescent** purine analogs will be able to provide useful information concerning the perturbation of nucleic acid structures.

L17 ANSWER 42 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 127:202400 CA
TI **Fluorescence** assays for **DNA** cleavage

AU Lee, S. Paul; Han, Myun K.
CS Department of Biochemistry, Georgetown University Medical Center, Washington, DC, 20007, USA
SO Methods in Enzymology (1997), 278(Fluorescence Spectroscopy), 343-363
AB Cleavage and joining reactions of nucleic acids are important processes in cellular events such as replication, recombination, and repair of **DNA**. Nucleic acids are readily cleaved by a variety of enzymes that recognize **DNA** sequences either specifically or nonspecifically. The efficiency of enzymic cleavage processes can be detd. by numerous methods such as gel electrophoresis, thin-layer chromatog., elution of the products from a DEAE-cellulose filter, and UV absorbance following HPLC to monitor disappearance of substrate or appearance of product. Current methods, though are either time-consuming or laborious. This work discusses the use of **fluorescence** spectroscopic approaches to design **fluorescence** assay systems monitoring enzyme-catalyzed **DNA** cleavage reactions. These assays are based on either FRET (**fluorescence** resonance energy transfer) or a **non-FRET** quenching mechanism.

L17 ANSWER 49 OF 183 MEDLINE on STN
AN 97215771 MEDLINE
TI Absorption, emission, and chiroptical spectra of neurokinin 1 tachykinin receptor antagonists: the role of charge-transfer states on the biological activity.
AU Pispisa B; Cavalieri F; Venanzi M; Sisto A
CS Dipartimento di Scienze e Tecnologie Chimiche, Universita' di Roma, Tor Vergata, Italy.
SO Biopolymers, (1996) 40 (5) 529-42.
AB A spectroscopic investigation, based on both electronic absorption and emission spectra as well as on chiroptical data, was performed on novel neurokinin 1 (NK1) tachykinin receptor antagonists, exhibiting interesting biological activity. These pseudopeptides have two fluorophores, i.e. indole (I) and naphthalene (N), and a central scaffold with different conformational mobility. Absorption spectra in methanol show the presence of a new band with respect to the sum spectrum of the isolated chromophores at around 285 nm, the intensity of which linearly increases as the bioactivity increases. This absorption disappears by using dioxane as solvent. It is ascribed to an intramolecular I-N charge-transfer (CT) complex that forms to different extent, depending on the flexibility of the scaffold. Under this condition, the molecules fold and apparently attain the correct conformation for competing substance P binding to the NK1 receptor, lending plausibility to the role of dipolar charged, spatially close aromatic moieties as topochemical elements in the mechanism of action of substance P antagonists. The excited-behavior parallels that in the **ground state**, as the **quenching** of the singlet state at 340 nm is found to be linearly dependent on the biological activity, too. Upon decreasing solvent polarity (methanol vs dioxane) the emissions of the dipolar state at around 370 nm disappears, while exciplex emission in the range of 400-500 nm occurs. This transition from charge-separated to exciplex-like states by lowering the dielectric constant of the medium very likely reflects a change in the structural features of the intramolecular I-N stacked complex, from a twisted or an asymmetrically overlapped conformation of the indolyl and naphthyl rings to a face-to-

face geometry. Implications of the rigidity of the molecules, arising from the formation of the intramolecular CT complex, on the ellipticity are briefly discussed.

L17 ANSWER 58 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 124:56650 CA
TI Synthesis and biological evaluation of novel NK-1 tachykinin receptor antagonists: the use of cycloalkyl amino acids as a template
AU Sisto, Alessandro; Bonelli, Fabio; Centini, Felice; Fincham, Christopher I.; Potier, Edoardo; Monteagudo, Edith; Lombardi, Paolo; Arcamone, Federico; Goso, Cristina; et al.
CS Chemistry Dep., Rome, Italy
SO Biopolymers (1995), 36(4), 511-24
AB In the course of a program aimed at synthesizing novel, potent NK-1 tachykinin receptor antagonists, a bioactive model was developed by comparing the low energy structures of a series of **peptide** and nonpeptide Substance P antagonists. The comparison was based on the superimposition of the arom. rings, assuming that the rest of the mol. behaves predominantly as a template to arrange the key arom. groups in the right spatial position. A series of 2-aminocyclohexanecarboxylic acid analogs was then selected as the best templates for reproducing the postulated bioactive structure, leading to several pseudo-**peptides** with interesting biol. activity. According to the mol. modeling, these compds. exhibit a neat parallel facing of the indolyl and naphthyl groups at about 3 Å distance. UV absorption and steady state **fluorescence** measurements support this conclusion, showing a linear correlation between the spectral properties and the binding affinity of these analogs. Stacking of the indole ring with naphthalene gives rise to a complex characterized by a well-defined molar extinction coeff. Consistently, steady state and lifetime **fluorescence** measurements suggest that the **quenching** process is ascribable to **ground-state** interactions between the chromophores. Implications of the π stacking propensity of arom. groups in the biol. activity of the compds. examd. are briefly discussed.

L17 ANSWER 59 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 123:105487 CA
TI Kinetic Studies by **Fluorescence** Resonance Energy Transfer Employing a Double-Labeled **Oligonucleotide**: Hybridization to the **Oligonucleotide** Complement and to Single-Stranded **DNA**
AU Parkhurst, Kay M.; Parkhurst, Lawrence J.
CS Department of Chemistry, University of Nebraska Lincoln, Lincoln, NE, 68588-0304, USA
SO Biochemistry (1995), 34(1), 285-92
AB A single 16-base oligodeoxyribonucleotide was labeled at the 3'-end with fluorescein and at the 5'-end with x-rhodamine (R*oligo*F); the chromophores served as a donor/acceptor pair, resp., for Forster resonance energy transfer. We exploited the striking differences in the steady-state emission spectra of the R*oligo*F as a single strand and in a duplex structure to signal hybridization in soln. and to det. the kinetics of duplex formation as the probe bound to its oligomer complement and to its target sequence in M13mp18(+) phage **DNA**. The binding followed second-order kinetics; in 0.18 M NaCl (pH 8) with 25%

formamide, the rate const. for binding to the oligomer complement was 5.7 x 105 M-1 s-1, and that to M13mp18(+) was 5.7 x 104 M-1 s-1. The source of the 10-fold decrease in the rate of binding to M13mp18(+) was examd. to differentiate between multiple nonproductive nucleation and rapid fluctuations in the structure around the target site. From simulations based on each model combined with assocd. exptl. results, we concluded that the slower binding was due to rapid structural fluctuations around the target site, with an effective target concn. 0.1 of that of the total. Comparisons of total fluorescein emission derived from both steady-state and lifetime measurements suggest that the 5'-x-rhodamine induces a conformational change that affects the interaction at the 3'-end between the fluorescein and the polymer. The effects of salt on the **fluorescence** were complex. The **static quenching** of fluorescein in the single-labeled, single-stranded **oligonucleotide** did not change with NaCl (0-0.18 M), whereas there were marked changes in the double-labeled probe, showing that the conformational effects mediated by the 5'-x-rhodamine were salt dependent.

L17 ANSWER 61 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 122:127871 CA
TI Quenching-resolved emission anisotropy: a steady state **fluorescence** method to study **protein** dynamics
AU Lakos, Zsuzsa; Szarka, Agnes; Koszorus, Laszlo; Somogyi, Bela
CS Department of Biophysics, Medical University School of Pecs, P.O. Box 99, Pecs, H-7601, Hung.
SO Journal of Photochemistry and Photobiology, B: Biology (1995), 27(1), 55-60
AB **Fluorescence** techniques can be used to obtain information about biol. objects in a non-destructive manner. One of these techniques is **fluorescence** quenching which involves a decrease in the **fluorescence** emission of a biol. object by externally added quenchers. Quencher mols. produce two kinds of **quenching**: **static** and dynamic. **Static quenching** occurs due to encounter pair formation between quencher and fluorophore mols., while dynamic quenching requires bimol. collisions. Unless one of the mechanisms can be neglected, steady state quenching expts. cannot provide information on the contributions of the two processes. However, time-resolved expts. are sensitive only to the dynamic process, and thus provide selective information about the relative motion of the quencher and fluorophore. Since the two quenching events are controlled by different physicochem. parameters, it is necessary to resolve them. In this paper, we describe a steady state method to resolve the **static** and dynamic **quenching** consts. (rather than time-resolved techniques). Our method is based on the simultaneous detn. of the **fluorescence** intensity and emission anisotropy data and can be regarded as the further development of quenching-resolved emission anisotropy (QREA). Since the steady state anisotropy and **fluorescence** lifetime are inversely related, by detg. the steady state **fluorescence** anisotropy, changes in the **fluorescence** lifetime (and hence the dynamic quenching process) can be monitored (if other parameters influencing the anisotropy remain const.). We present a theor. description of the method, computer simulations testing its accuracy and results of model expts. with pyridoxamine-phosphate-labeled lysozyme and acrylamide. By changing the external viscosity, we obtained data on the theor. inverse

relationship between the dynamic quenching const. and viscosity. The application conditions are also discussed.

L17 ANSWER 111 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 108:52042 CA
TI Accessibilities of the sulfhydryl groups of native and photooxidized lens crystallins: a **fluorescence** lifetime and quenching study
AU Andley, Usha P.; Clark, Barbara A.
CS Harvard Med. Sch., Massachusetts Eye Ear Infirm., Boston, MA, 02114, USA
SO Biochemistry (1988), 27(2), 810-20
AB **Fluorescence** lifetime and acrylamide quenching studies on the N-(iodoacetyl)-N'-(5-sulfo-1-naphthyl)ethylenediamine (1,5-IAEDANS)-labeled SH groups of bovine lens α-, βH-, and γ-crystallins were carried out to characterize the microenvironment of the SH groups and the changes produced by singlet O-mediated photooxidn. For the untreated **proteins**, the lifetimes of the major decay component of the **fluorescence**-labeled crystallins were 15.2, 14.4, and 13.0 ns, and the quenching rate const. (kq) values were 16.6 × 107, 26.9 × 107, and 32.7 × 107 M-1 s-1 for α-, βH-, and γ-crystallins, resp. Thus, as the polarity of the SH site increased (i.e., its lifetime decreased); its accessibility to collisional quenching by acrylamide also increased. The minor decay component of the **fluorescence** label was not significantly quenched by acrylamide for all 3 classes of crystallins. When the **proteins** were irradiated in the presence of methylene blue in a system generating singlet O, the kq value for acrylamide quenching of the major decay component of α-crystallin decreased to zero, while its lifetime decreased to 6 ns. Neither the lifetime nor the kq of α-crystallin recovered completely in the presence of the singlet O quencher, Na azide. Light-induced binding of the photosensitizer, methylene blue, to the crystallins was obsd. by absorption spectroscopy. The bound photosensitizer partially quenched the **fluorescence** lifetime of the N-acetyl-N'-(5-sulfo-1-naphthyl)ethylenediamine (AEDANS) label in irradiated α-crystallin. A further decrease in the lifetime occurred as a result of the singlet O-mediated conformational change. Apparently, the **fluorescence** lifetime of the AEDANS is fully quenched in the irradiated α-crystallin and there is no further quenching by acrylamide. An increase in the fraction of the minor component of βH-crystallin which was inaccessible to acrylamide quenching was obsd. after irradn. There was no effect of irradn. on the kq for acrylamide quenching of the major component of the decay of AEDANS bound to βH- or γ-crystallins. **Static quenching** was found to contribute significantly to the steady-state quenching plots of the polar SH sites of irradiated α-crystallin and of untreated and irradiated βH- and γ-crystallins, but it had no detectable role in the case of untreated α-crystallin. **Fluorescence** anisotropy of the AEDANS label bound to the crystallins was higher in the irradiated crystallins as compared with the controls.

L17 ANSWER 122 OF 183 CA COPYRIGHT 2005 ACS on STN
AN 107:111722 CA
TI Novel approaches towards characterization of the high-affinity nucleotide binding sites on mitochondrial F1-ATPase by the **fluorescence**

probes 3'-O-(1-naphthoyl)adenosine di- and triphosphate
AU Weber, Joachim; Roegner, Matthias; Schaefer, Guenter
CS Inst. Biochem., Med. Univ. Luebeck, Luebeck, D-2400/1, Fed. Rep. Ger.
SO Biochimica et Biophysica Acta, Bioenergetics (1987), 892(1), 30-41
AB The fluoroscence properties of 3'-O-(1-naphthoyl)adenosine di- and triphosphates (N-ADP and N-ATP, resp.) were investigated in detail. Of special importance for the use of these analogs as environmental probes is their high quantum yield (0.58 in H2O) and the polarity dependence of shape and wavelength position of the emission spectrum. Upon binding of N-ADP and N-ATP to mitochondrial F1-ATPase, the **fluorescence** intensity is markedly decreased, due to polarity changes and **ground-state quenching**. By using this signal for equil. binding studies, 3 (at least a priori) equiv. nucleotide-binding sites were detected on the enzyme. The intrinsic dissocn. consts. are as follows: N-ADP/Mg2+ 120 nM; N-ATP/Mg2+ 160 nM; N-ADP/EDTA 560 nM; N-ATP/EDTA 3500 nM. For bound ligand, the environment was rather unpolar; the rotational mobility of the fluorophore is restricted, its accessibility for I- (as a quencher) is hindered. These facts suggest the binding sites are quite deeply embedded in the **protein**. The conformation of the binding domains is strongly dependent on the absence or presence of Mg2+, as can be seen from the relative efficiencies of the singlet-singlet energy transfer from tyrosine residues in the **protein** to bound naphthoyl moieties. Investigation of the binding kinetics revealed this process as biphasic (in presence of Mg2+). After the 1st fast step (rate of binding kon >1 × 106 M-1 s-1), in which the analog is bound to the enzyme, a slow local conformational rearrangement occurs.

=> log y
STN INTERNATIONAL LOGOFF AT 13:56:30 ON 15 OCT 2005